

Claims

*Sub 1*

1. Method for enzymatic preparation of homogentisate (HMO) from 4-hydroxypyruvate (HPP), characterized in that it consists in carrying out, in a suitable reaction medium, the following enzymatic reactions:

- enzymatic conversion of HPP into 4-hydroxyphenyl-acetate (HPA) with a first suitable enzyme, then
- enzymatic conversion of HPA into HMO with a second suitable enzyme.

2. Method according to Claim 1, characterized in that the first enzymatic conversion is catalysed by a suitable HPP-oxidase.

*Sub D1*

3. Method according to Claim 2, characterized in that the HPP-oxidase originates from bacteria which can grow on HPP as the only carbon source.

4. Method according to Claim 2, characterized in that the HPP-oxidase originates from *Arthrobacter*.

5. Method according to Claim 1, characterized in that the second enzymatic conversion is catalysed by a suitable HPA-hydroxylase.

*Sub D1*

6. Method according to Claim 5, characterized in that the HPA-hydroxylase originates from bacteria which can grow on HPA as the only carbon source.

7. Method according to Claim 6,  
characterized in that the bacteria are chosen from  
*Pseudomonas acidovorans*, *Xanthobacter*, *Pseudomonas*  
*alcaligenes*, *Flavobacterium sp.*, *Bacillus subtilis*,  
5 *Nocardia sp.* DM1 and *Rhodococcus erythropolis*.

8. Method according to Claim 5,  
characterized in that the HPA-hydroxylase is extracted  
from *Pseudomonas acidovorans*.

9. Method according to one of Claims 1 to  
10 8, characterized in that both enzymatic reactions are  
carried out in the same reaction medium containing HPP,  
the two suitable enzymes being present together at the  
same time in the reaction medium.

10. Method according to one of Claims 1 to  
15 9, characterized in that the two suitable enzymes are  
introduced into the suitable reaction medium in the  
form of protein extracts, or alternatively they can be  
produced in situ by suitable biological organisms.

20 11. Method according to one of Claims 1 to  
10, characterized in that it is carried out in the  
presence of an HPPD inhibitor in the suitable reaction  
medium.

add C2